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Microalgae for a macroenergy world

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ABSTRACT

One of the most important dilemmas of the modern world is to supply enough energy with minimal environmental impact. On this demand bioenergy from renewable biofuels is of growing public and private interest.

Recent developments in the scientific researches show that microalgae have potential as a source of bioenergy. With their exception of being one of the oldest residents of the Earth and playing a vital role in building up the atmosphere, microalgae have a variety of diversified strains, biochemical routes and products that can be used for biofuel processing. An increasing number of researchers, academics, entrepreneurs and investors are now working on new technologies to adapt microalgae originated energy into our daily life.

The aim of this review is to focus on microalgae based biofuels under the main titles of biodiesel, biohydrogen, bioethanol and biomethane.

For evolution in bioenergy that started with the first generation way through the third generation and today stepping on the concept of fourth generation, microalgae will be a good candidate for an alternative energy source.

Contents

1. Introduction

Today main streams of energy consumption can be listed as electricity generation, industrial activities, transportation, commercial and residential needs. This energy demand is supplied from resources such as coal, natural gas, nuclear power, liquid fossil fuels and renewables (mainly hydropower). According to the projections, world marketed energy consumption will increase up to 739 quadrillion BTU by the year 2035 which exceeds the value of year 1990 by twofolds [1]. Even with some deviations these projections show the massive energy need for the future, in other words the importance of using each and every energy source.

With the approach of the second millennium, concerns about environment related with pollution and global warming started to

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increase the pressure on the societies. On the other hand rapid declines in global petroleum reserves and escalating prices have made it obligatory to move towards the development of alternative energy sources.

In the search of new energy substitutes carbon neutral, cost-effective, sustainable and environmentally friendly renewable fuels from biological resources have received the main attention. Microalgae can be a good example for a renewable energy source that holds a potential without an adverse effect on the supply of food and other crop products [2–4].

Microalgae are photosynthetic, free floating microorganisms that can form filaments and colonies which have high ability for the adaptation even to extreme ecological habitats. They are able to convert light and carbon dioxide through cellular activities to produce special chemicals like carbohydrates, proteins, lipids, vitamins and pigments [2–4]. Contemporary commercial microalgae production depends on these chemicals that have application in cosmetic, health food, feed supplement, chemical and pharmaceutical industries (Table 1).

Even if the main focus is still on the biochemical products, the concept of using microalgae as a source of fuel is starting to catch attention. The know-how from the commercial production constructs the basis for the progress that encouraged the researchers in the universities and institutes to put more effort into developing the microalgal energy production systems. A good indication of this interest can be seen from the academic researches considering bioenergy from microalgae. The number of published papers about microalgae from different countries referenced in Science Citation Index has a clear acceleration with the turn of the 1990s (Fig. 1). At a first glance the reason of this acceleration can be grounded on the fact of spread internet use, more publishing journals and better interaction between researchers all around the globe. On the other hand in the last decade the increase in the number of companies, up to 200, involved in producing fuel from algae is the viable sign of this effort and interaction (Table 2). As can be seen from the top 10 countries considering the number of publications and companies there is a clear relation (Fig. 1) which is important for the realization of the scientific developments to leap to the commercial scale [5,6].

Today global awareness of the societies has been kept alive with the bombardment of news about biofuels. It became ordinary to hear announcements about microalgal biofuels with fancy slogans like saving the world. But because not much was done compared to the talks, microalgal biofuels are losing ground. The motivation of this review is to give detailed background about the works that has been done with microalgae to obtain biofuels with biochemical pathways through the years and summarize them according to the biofuel type even in a limited space of a review text.

2. Biofuels from microalgae

When processed through chemical or biological reactions, microalgae can provide different types of renewable biofuels (Fig. 2). These include biodiesel, biohydrogen, bioethanol and biomethane. With regard to microalgae based fuels the main focus is on the biodiesel production. Biohydrogen production is also popular with its potential in modern applications like fuel cells. The other two, bioethanol and biomethane, are considered as a part of integrated processes.

2.1. Biodiesel

Technically biodiesel is an alternative transportation fuel based on monoalkyl ester building blocks of long-chain fatty acids that is produced commercially from some common vegetable sources including soy, sunflower, safflower, canola, and palm [7,8].

Because of the growing public deprecation about the use of food crops for fuel production, researchers have turned their focus to alternative, non-food related substitutes such as microalgae [7].

Microalgae can form diverse kinds of cellular lipids including neutral lipids, polar lipids, wax esters, sterols and hydrocarbons, besides prenyl derivatives such as tocopherols, carotenoids, terpenes, quinones and phytylated pyrrole derivatives such as chlorophylls [9].

Compared to vegetables these lipids have diversified fatty acid compositions with higher unsaturation levels. The size of the fatty acid chains and their level of unsaturation play an important role in the quality of the biodiesel. High quality biodiesel should have low temperature performance and oxidative stability. These two constraints can be supplied by low concentrations of both

Table 1
Commercial products from microalgae (OP: open ponds; PBRs: photobioreactors; P: phototrophic; H: heterotrophic)..*Source*: Adapted from Refs. [2,135–138]

Microalgae	Product	Production	Mode	Application
Spirulina platensis	Phycocyanin, biomass	OP, PBRs	P	Health food, cosmetics
Chlorella vulgaris	Lipid, biomass	OP, PBRs	P,H	Health food, food supplement, feed
Dunaliella salina	Carotenoids, β-carotene	OP, PBRs	P,H	Health food, food supplement, feed
Lyngbya majuscule	Immune modulators	OP, PBRs	H	Pharmaceuticals, nutrition
Haematococcus pluvialis	Carotenoids, astaxanthin	OP, PBRs	P,H	Health food, pharmaceuticals, feed additives
Monodus subterraneus	Eicosapentaenoic acid	OP, PBRs	P	Pharmaceuticals, nutrition
Odontella aurita	Fucoxanthin, fatty acids	OP	P	Pharmaceuticals, cosmetics, baby food
Porphyridium cruentum	Polysaccharides	PBRs	P,H	Pharmaceuticals, cosmetics, nutrition
Aphanizomenon flosaquae	Glycoproteins, vitamins, lipids	OP, PBRs	P	Pharmaceuticals, cosmetics, nutrition, nurition
Isochrysis galbana	Fatty acids	OP, PBRs	P,H	Animal nutrition
Phaedactylum tricornutum	Lipids, fatty acids	OP, PBRs	P,H	Pharmaceuticals, nutrition
Euglena gracilis	α-Tocopherol, biotin	PBRs	P,H	Pharmaceuticals, nutrition
Nitzchia laevis	Eicosapentaenoic acid	PBRs	H	Pharmaceuticals, nutrition
Crypthecodinium cohnii	Docosahexaenoic acid	PBRs	H	Pharmaceuticals, nutrition
Chlorella protothecoides	Biomass, lipids, tocopherol	PBRs	P,H	Pharmaceuticals, nutrition
Galdieria sulphuraria	C-phycocyanin	PBRs	P,H	Health food, cosmetics
Aphanizomenon flos-aquae	Vitamins, fatty acids, phycocyanin	OP, PBRs	P	Pharmaceuticals, nutrition
Shizochytrium sp.	Docosahexaenoic acid	PBRs	H	Pharmaceuticals, nutrition
Chlorella minutissima	Eicosapentaenoic acid	PBRs	P,H	Pharmaceuticals, nutrition
Prototheca moriformis	Vitamin C	PBRs	H	Pharmaceuticals, nutrition
Parietochloris incise	Arachidonic acid	PBRs	P	Pharmaceuticals, nutrition
Tetraselmis suecica	Lipids, PUFA,	OP, PBRs	P,H	Pharmaceuticals, nutrition
Nannochloropsis oculata	Lipids	OP, PBRs	P,H	Pharmaceuticals, cosmetics, nutrition

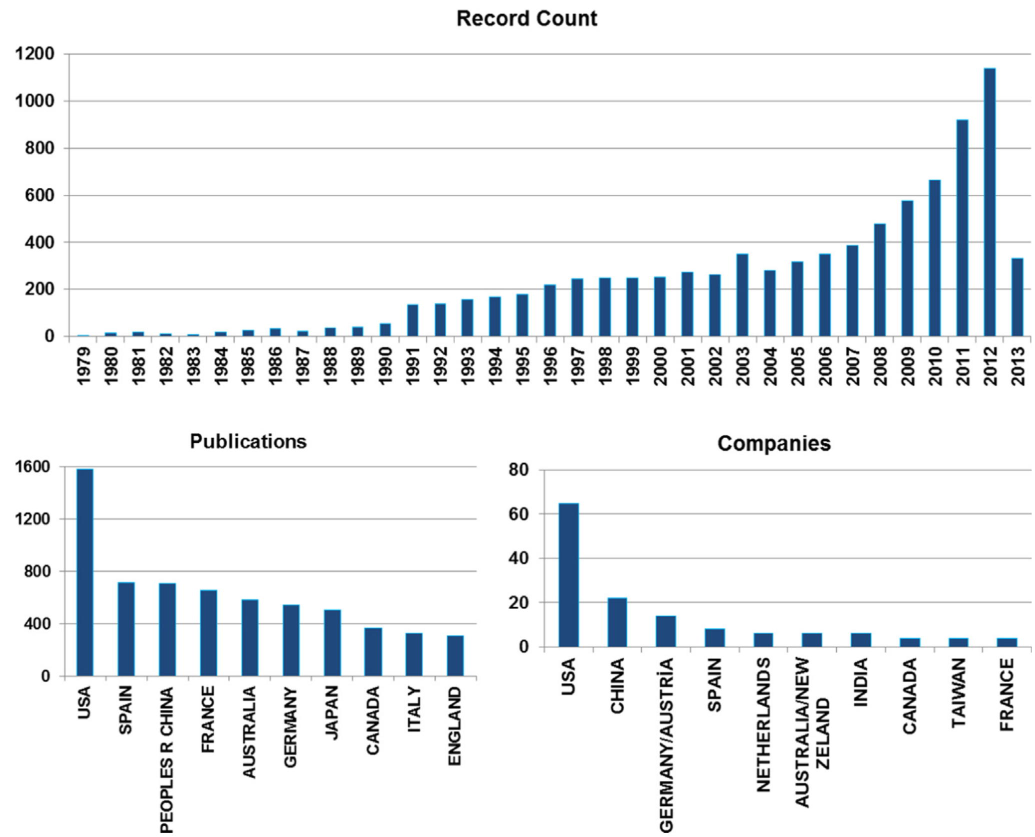


Fig. 1. Annual publication count and the top 10 countries considering the number of publications and companies.
Source: Adapted from Refs. [139–142,198].

Table 2
List of companies working on microalgal biofuel technologies..*Source*: Adapted from Refs. [139–142]

A2Be Carbon Capture	Algae Venture Systems	Algaewheel	AlgaEnergy
AlgoDyne Ethanol Energy Corp	Aquaflow Bionomic	Aquatic Energy	Aurora Algae, Inc.
Algasol	Algenol	Amyris Biotech	Algaecake Tech. Corp.
AXI	Algafuel	Algae Biotech SA	Algae Link N.V
Airbus	Air New Zealand	Boeing	BioAlgaene LLC
Blue Marble Energy	Bodega Algae	BBI	Bionavitas
BTR Labs	Biofuel Systems	Biomara	Bisantech Nuova GmbH
Continental Airlines	Cequesta Algae	Carbon Capture Corporation	Cellana—Shell & HR Biopetroleum
Chevron	Culturing Solutions, Inc	Clean Algae SA	Circle Biodiesel & Ethanol Corporation
Community Fuels	Canadian Pacific Algae	Center of Excellence for Hazardous Materials Management	Dynamic Biogenics
Diversified Energy	DFI Group	ENEL	EADS and IGV GmbH
Enhanced Biofuels & Technologies	Fluid Imaging Technologies	General Atomics	Green Gold Algae and Seaweed Sciences Inc.
General Electric	GreenFuel Technologies Corporation	Greenshift	Green Star Products, Inc.
Greenbelt Resources Corporation	Global Green Solutions	Hawaiian Electric Company	HR Biopetroleum
Inventure Chemical Technology	Ingrepo	Infinifuel Biodiesel	International Energy
Imperium Renewables	Jet Blue	Japan Air	Kelco
KuehnleAgrosystems	Kai Bioenergy	KLM Airlines	LiveFuels
LS9 Synthetic Bioenergy solutions	MBD Biodiesel	Neptune	Neste Oil
Northington Energy	Organic Fuels	OriginOil	Ocean Technology & Environmental Consulting
Oilfox Argentina	Petro Algae	Phycal	Pure Power Energy
PetroSun Biofuels	Phyco2	Planktonix Corporation	Proviron
Renewable Energy Group	Revolution Biofuels	SeaAg	SBAE Industries
Sunx Algae Oil Research Lab	Solena Group	Sapphire Energy	Seambiotic
Solazyme	SunEco	Sunrise Ridge	Solix Biofuels
Solray	Synthetic genomics and Exxon Mobil Corp.	SGC Energia SGPS S.A.	SAIC Corp.
Texas Clean Fuels	Virgin Airways	Valcent	VG energy
W2 Energy	XL Renewables—Sigmae		

long-chain saturated and poly-unsaturated fatty acid oils [10–12]. Fatty acid profiles of the microalgae depend on the species and culture conditions. During optimum culture conditions microalgae synthesize fatty acids (up to 20% of dry cell weight), mainly for esterification into glycerol based membrane lipids. But when faced with stress conditions microalgae can diverge from lipid production pathway towards the synthesis and accumulation of neutral lipids that may reach up to 50% dry cell weight, mainly in the form

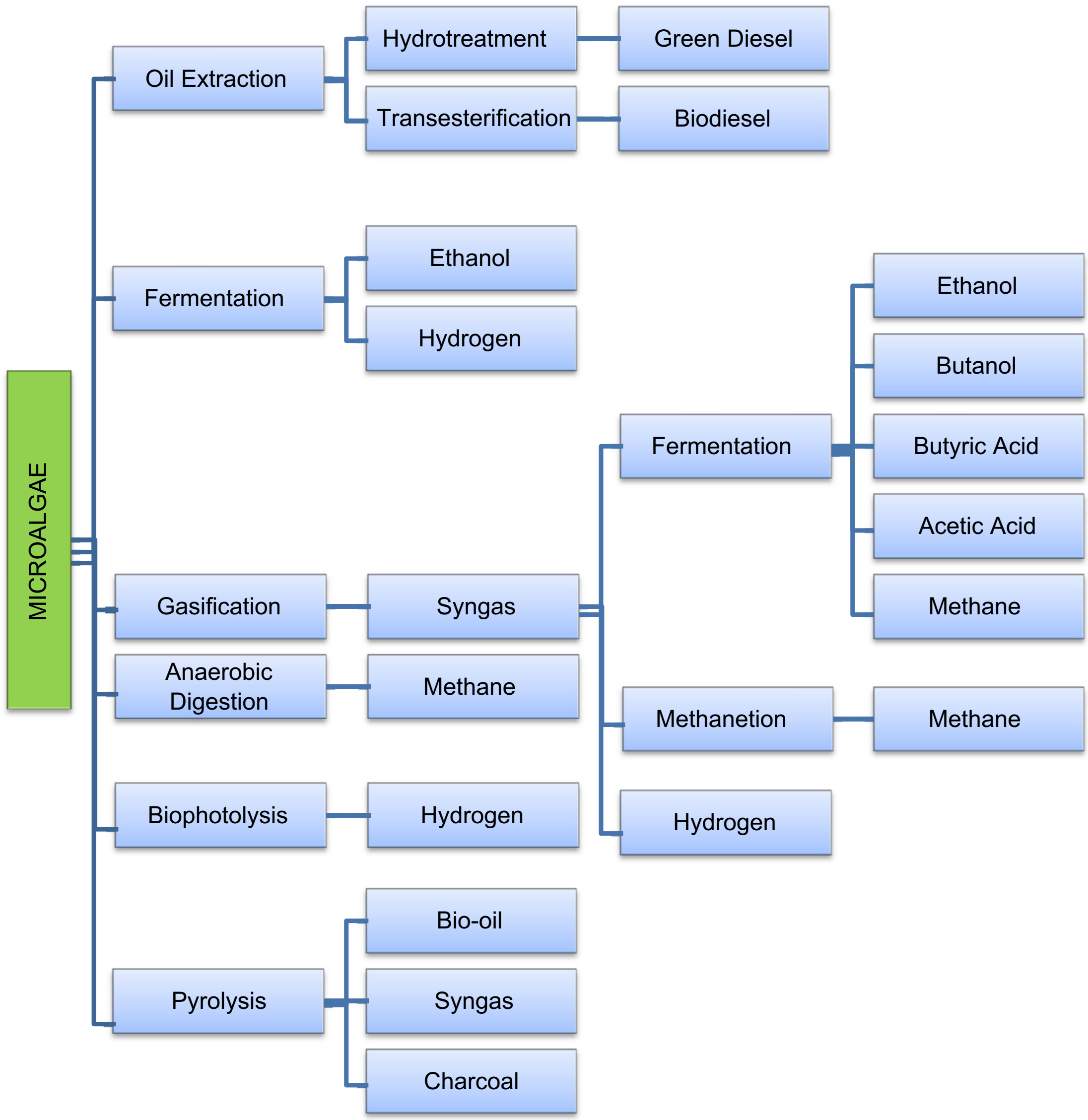


Fig. 2. Biofuel routes from the standpoint of microalgae.
Source: Adapted from Refs. [8,16,108,199].

of triacylglycerols which are actually the key component of algal lipids for biodiesel production. Triacylglycerols are distinct from membrane glycerolipids because of their character as the storage material for energy instead of structural usage [9,13].

Lipid productivity of a microalga is accepted to be an indicator of its potential for biodiesel production.

Studies focus on different parameters, such as nutrient source, nutrient concentration, light intensity, salinity, pH, mixing speed and temperature, to enhance the lipid productivity of the microalgae (Table 3). Microalgae show variable responses to the effects resulting in various lipid productivities. For example on cultivation with 10% CO_2, biomass productivity of the *Scenedesmus* sp. was higher than *Botryococcus branuii*; on the other hand lipid productivity was lower but similar productivities could be seen in the case of flue gas (5.5% CO_2) cultivation [14]. In another study, results indicated that potassium phosphate and magnesium sulfate are the major media components affecting the lipid productivity of *Botryococcus branuii* and the lipid and biomass optimized media should differ in concentrations [15]. The key is to select the proper method of approach, considering the microalga, for the lipid productivity.

Extraction of microalgal lipids is the bottleneck for high yield in productions. After the harvest, the culture starts its downstream journey. As a first step the excess water that is an unwanted volumetric load is removed through concentration processes like filtration, drying, flocculation and centrifugation. The concentrated cells can either be extracted directly or through a disruption step in which the intercellular content was released to enhance the extraction process. Considering microalgae because the target product, oil, is trapped inside strong cell walls, the disruption step can be termed as a part of the extraction step [16–18]. Microalgal oils could be extracted using milling, expeller press, high pressure homogenization, solvent, enzyme, supercritical fluid, osmotic shock, pulsed electric field, microwave or ultrasound techniques. The selection of the method depends on the specie, cost and efficiency as well as the environmental concerns. The extracted lipids then passed to the fractionation step where the undesired polar lipids and non-acylglycerol neutral lipids (such as free fatty acids, hydrocarbons, sterols, ketones, carotenes, and chlorophylls) were removed. Later the purified product is converted into biodiesel through the transesterification process [19–24].

Table 3
Various studies focusing on lipid productivities as the key element for biodiesel production.

Microalgae	Effect	Time	Cultivation	PBR type	Light intensity (μE m^{-2} s^{-1})	Temp. (°C)	Medium	Maximum production	Ref.
Botryococcus braunii (UTEX 572)	CO_2	14 days	Batch–autotrophic	Bubble column	150	25 ± 1	Modified Chu 13	• 5.51 ± 1.53 mg L^{-1} d^{-1} (21% biomass) with 10% CO_2 enriched air • 20.65 mg L^{-1} d^{-1} (24%) with 5.5% CO_2 containing flue gas	[14]
Botryococcus braunii (765)	CO_2	28 days	Batch–autotrophic	Airlift (3 L)	150 ± 10	25	Modified BG11	11.74 mg L^{-1}d^{-1} (12.71 ± 0.83% biomass) with 20% CO_2 enriched air	[143]
Botryococcus braunii (UTEX 572)	Media components	18 days	Batch–autotrophic	Bubble column (0.6 L)	50	22	Modified BG11	• 0.19 g L^{-1} d^{-1} (64.96% biomass) in lipid optimized media • 0.18 g L^{-1} d^{-1} (59.56% biomass) in growth optimized media	[15]
Botryococcus braunii (KMITL 2)	Light intensity, light cycle, nitrogen, phosphorus, iron, cultivation time, salinity	40 days	Batch–autotrophic	Flasks (1 L)	0–538 (L:D cycle)	25	Chlorella	54.69 ± 3.13% with 200 μE m^{-2} s^{-1} continuous light, 222 mg L^{-1} phosphorus and a salinity of 0 psu	[144]
Chlamydomonas reinhardtii	pH and CO_2	31 days	Batch–autotrophic	BIOCOIL (15 L)	220	25 ± 1	Artificial or waste water	0.505 ± 0.02 g L^{-1} d^{-1} (25.25% biomass) with 33% CO_2 at pH 7.5	[145]
Chlorella vulgaris (CCAP 211)	Temperature, nitrogen concentration and extraction techniques	14 day	Batch–autotrophic	Erlenmayer flask (2 L)	70	15–25	Guillard F2	• 20.22 ± 0.60 mg L^{-1} d^{-1} (14.71 ± 0.30%) at 25 °C • 20.30 ± 0.40 mg L^{-1} d^{-1} (15.31 ± 0.51%) with 0.375 g L^{-1} nitrogen concentration	[146]
Chlorella sorokiniana (GXNN01)	Carbon sources and concentrations	70 h	Batch–autotrophic, heterotrophic	Erlenmayer flask (0.15 L)	0–80	30 ± 2	BBM	• 0.288 ± 0.008 g L^{-1} (0.287 ± 0.018 g g_{DW}^{-1}) with acetate	[147]
Chlorella minutisima (UTEX LB-2341)	Long term outdoor production	80 days	Batch or perfusion; autotrophic–mixotrophic	Cylindrical vessel (20 L × 10; 200 L total)	0–700	30–35	Enriched sea water	• Glucose concentration of 20 mmol L^{-1} gave the maximal lipid yield • 0.048% lipid g_{DW}^{-1} day^{-1} (%23.2) in mixotrophic perfusion (rate, 2.8 L h^{-1}) culture	[148]
Chlorella protothecoides (UTEX 249)	Carbon source concentration, nitrogen concentration, salinity, pH level and agitation speed	103 h	Batch–autotrophic, mixotrophic, heterotrophic	Erlenmeyer flasks (0.1 L)	8W	26	Modified basal	• 0.25 g L^{-1} d^{-1} (25.25 ± 0.07%) with 15 g L^{-1} glucose, 6.9 pH • 0.19 g L^{-1} d^{-1} (20.33 ± 5.13%) with 20.4 gL^{-1} glycerol, 7.1 pH • 0.17 ± 0.01 g L^{-1} d^{-1} (23.08 ± 3.18%) with 20.5 g L^{-1} acetate, 6.7 pH	[149]
Chlorella protothecoides (SAG 33.80)	CO_2	14–15 days	Batch–autotrophic, mixotrophic	Erlenmeyer flasks (0.25 L)	100 ± 10	24 ± 1	BG11 with Peptone	• 21.3 ± 2.5% DW (Mixotrophic, 1% glycerol) • 11.5 ± 3.1% DW (Autotrophic) • 35.8 ± 1.5% DW (Mixotrophic, N-deprived)	[150]
Chlorella pyrenoidosa	Waste water	10 days	Batch–Mixotrophic	Flasks (0.1 L)	63	25–27	Diluted piggery waste water	(with 1000 mg L^{-1} COD)	[151]
Chlorella pyrenoidosa FACHB-9	Waste water	120 h	Batch/fed batch–mixotrophic	Flasks (0.5 L)	40.5 (14:10 L/D cycle)	27 ± 1	Soybean process waste water	0.4 g L^{-1} d^{-1} (37 ± 9.34% DW) with fed batch culture	[152]
Auxenochlorella protothecoides UMN280	CO_2, waste water	12 days	Batch–mixotrophic	Roux bottles (0.58 L)	60	25 ± 2	Waste water (0.5 L) and BG11 seed culture (0.08 L)	• 0.182 g L^{-1} (18.66% DW) without CO_2 • 0.418 g L^{-1} (20.82% DW) with 1% CO_2 • 0.516 gL^{-1} (20.58% DW) with 5% CO_2	[153]
Chlorella vulgaris	KNO_3, CO_2 and light intensity	250 h	Batch		24–120	25	Artificial sea water		[154]

Table 3 (*continued*)

Microalgae	Effect	Time	Cultivation	PBR type	Light intensity (μE m^{-2} s^{-1})	Temp. (°C)	Medium	Maximum production	Ref.
				Membrane sparged cylindrical vessel (5 L)				40 mg L^{-1} d^{-1} (~20%) at 60 μE m^{-2}s^{-1} light intensity, 1 mM KNO_3 concentration, 1% CO_2 enrichment	
Chlorella vulgarisKCT-CAG10032	CO_2	14 days	Batch–autotrophic	Bubble column	150	25 ± 1	BG11	6.91 ± 0.03 mg L^{-1}d^{-1} (7% biomass) with 10% CO_2 enriched air	[14]
Chlorella vulgaris BEIJ-H14	Glucose and urea	66.5 h	Fed batch–heterotrophic	Stirred tank (600 L)	Dark	36–37	Glucose enriched medium	9.7% dry weight	[155]
Chlorella vulgaris CCALA 256	Nutrient limitation	8 days	Batch–autotrophic	Thin layer (150 L)	100–960	19.5–33	1/4 SS	0.326 ± 0.010 g L^{-1} d^{-1} (30.6 ± 0.5% DW)	[156]
Choricystis minor	Temperature, dilution rate and postharvest methods		Continuous–autotrophic	Stirred tank (4 L)	550	10–30	BG 11	• 82 mg L^{-1} d^{-1} (21.3 ± 1.7%) at 25 °C and a dilution rate of 0.014 h^{-1} • Lipid content increased up to 59.5 ± 1.6% under postharvest conditions without phosphate and nitrate	[157]
Nannochloropsis oculata	Temperature, nitrogen concentration and extraction techniques	14 days	Batch–autotrophic	Erlenmayer Flask (2 L)	70	25–38	BBM	• 9.11 ± 0.30 mg L^{-1} d^{-1} (14.92 ± 0.82%) at 15 °C • 16.41 ± 0.11 mg L^{-1} d^{-1} (15.86 ± 0.59%) with 0.075 gL^{-1} nitrogen concentration	[139]
Nannochloropsis oculata	Temperature, nitrogen and yeast extract concentrations	16 days	Batch–photomixotrophic	Airlift (2 L)	160–270	15–35	F2	• 10–15% (50 ppm nitrogen) • 20-25% (12.5 ppm nitrogen) • 25–30% (15 °C) • 20–25% (35 °C)	[158]
Haematococcus pluvialis	Continuous light intensity or light cycles, no nitrogen or no aeration on production	14 days	Batch–autotrophic	Flask	90	24	BBM	• 15.61 ± 1.46% DW Under normal conditions • 34.85 ± 0.78% DW Under full medium, continuous light with aeration • 32.99 ± 2.77% DW, Under continuous light, aeration, no nitrogen	[159]
Neochloris oleoabundans (UTEX-1185)	Nitrogen sources and concentrations	7 days	Batch–autotrophic	Bubble column (1 L)	360	30 ± 2	SE	0.133 g L^{-1} d^{-1} (38%) with 5 mM sodium nitrate as nitrogen source	[160]
Neochloris oleoabundans (UTEX-1185)	Temperature, CO_2 and nitrate	18 days	Batch–autotrophic	Bubble column (1 L)	150	26–30	BM	56% biomass with nitrogen starvation at 30 °C temperature without CO_2 in air	[161]
Neochloris oleoabundans (UTEX-1185)	Nitrogen starvation	140 h	Continuous–autotrophic	Flat panel airlift (1 L)	270	25	BBM	• 126 g m^{-3} d^{-1} (23%) continuous culture with no mineral limitation with nitrate starvation	[162]
Scenedesmus sp. KCTC AG20831	CO_2	14 days	Batch–autotrophic	Bubble column	150	25 ± 1	BG11	• 20.65 ± 0.13 mg L^{-1}d^{-1} (9% biomass) with 10% CO_2 enriched air • 39.44 mg L^{-1} d^{-1} (18%) with 5.5% CO_2 containing flue gas	[14]
Tetraselmis suecica	Light and nitrogen concentration	9 days	Batch–autotrophic	Stirred tank (20 L)	36.3–133.1	20 ± 1	Modified F2	• 16–18% DW with nitrogen deplete two stage process • 10–12% DW with single stage process	[163]

The preferred route for biodiesel production from oils is transesterification rather than pyrolysis and micro-emulsion because of the cost and quality concerns [25]. Transesterification is simply the reaction between triacylglycerols and an acyl-acceptor which can be carboxylic acids (acidolysis), alcohols (alcoholysis) or another ester (interesterification) [25,26]. Biodiesel production from microalgal oils through transesterification process has several steps (Fig. 3).

The reaction can be chemically (acid or base) or biologically (enzyme) catalytic or non-catalytic (high pressure process) [27–29].

Homogeneous (NaOH, CH_3ONa, KOH, $KOCH_3$) and heterogeneous (alkaline earth metal oxides, zeolite, KNO_3 loaded on Al_2O_3, KNO_3/Al_2O_3, BaO, SrO, CaO, MgO) base catalysts are conventionally used for biodiesel production. They are more effective with an oil having free fatty acid concentration up to 2% resulting in high reaction rates (4000× faster) and conversion efficiency under lower operation temperatures compared to acid catalysts. But considering water content and acidity of the biodiesel feedstock acid catalysts are preferred to prevent saponification. Even if acid catalysts are effective with non-edible oils, their limitations like the need for higher amounts of alcohol, higher temperatures and pressures, slower reaction rates result in lower yields. Both homogeneous and heterogeneous acid catalysts (sulfuric acid, hydrochloric acid, phosphoric acid, and sulfonated organic acids) are used especially in two step biodiesel production where in the first step the oil reacts with alcohol by acid catalysts and later reacted with the base catalyst. This procedure is preferred with high free fatty acid concentrated oils, where the procedure decreases the value of the free fatty acids to operational levels [10,30].

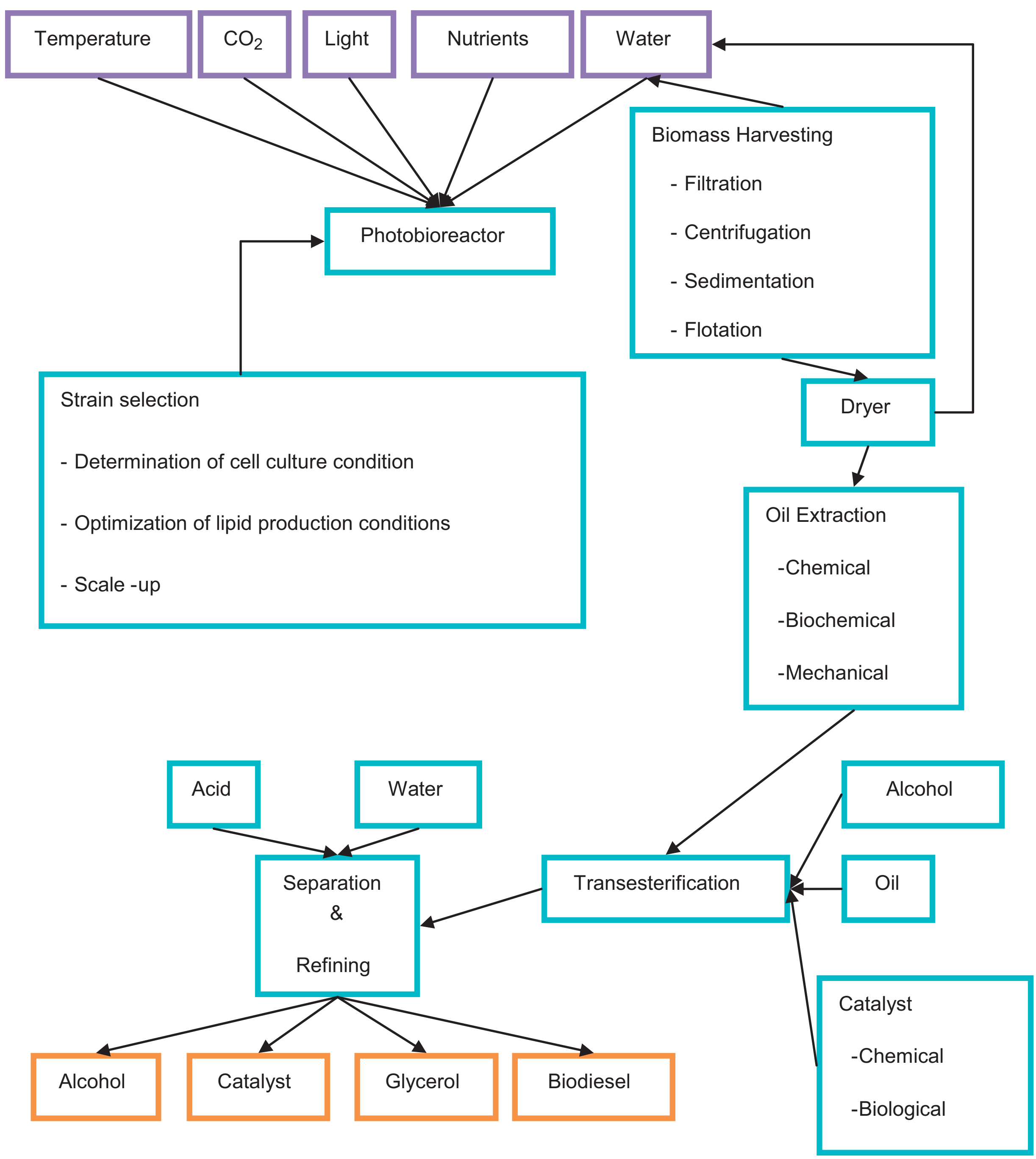


Fig. 3. Microalgal biodiesel production.
Source: Adapted from Refs. [7, 46,47,199,200].

Conventional technology of chemical catalysis has some constraints due to the environmental problems related with soap formation and energy demand due to the downstream processes to recover glycerol and catalysts [26,28,31].

Biological catalysis has some advantages over the chemical catalysis including lower energy demand, moderate reaction conditions, lower alcohol to oil ratio, easier product recovery and high conversion [31,32].

Lipases are the key biocatalysts that are used in biodiesel production. Major producer microorganisms for lipases are *Aspergillus niger*, *Aspergillus oryzae*, *Bacillus pumilis*, *Burkholderla cepacia*, *Candida antarctica*, *Candida cylindracea*, *Candida lipolytica*, *Candida paralipolytica*, *Candida rugosa*, *Chromobacterium viscosum*, *Enterobacter aerogenes*, *Eremothecium ashbyii*, *Escherichia coli*, *Geotrichum candidum*, *Mucor miehei*, *Penicillium cyclopium*, *Penicillium expansum*, *Penicillium restrictum*, *Pseudomonas cepacia*, *Pseudomonas fluorescens*, *Pseudomonas fragi*, *Rhizopus arrhizus*, *Rhizopus delemar*, *Rhyzomucor miehei*, *Rhizopus oryzae*, *Saccharomyces cerevisiae*, and *Thermomyces lanuginosus* which are also commercially important to meet the needs of the large scale biodiesel processes [33,34].

Main bottlenecks of this process include high cost of enzyme, low yield due to inactivation, longer reaction time, slower reaction rate and the amount of water and organic solvents in the reaction mixture [25,32].

The limitations in the biocatalysis are managed via the immobilization of the lipases in packed bed reactors to prevent washout and inactivation [35], using whole cell immobilization [26], ionic liquids in enzyme catalysis [31,36], genetic modifications and recombinant microorganisms to enhance the efficiency and the production of the enzyme [26], alternative acyl acceptors [37], stepwise processes [38,39], moderate polar solvents [40], non-catalytic methods under supercritical conditions [41,42] and high pressure processes to stabilize the enzymes [43,44].

From the economical point of view which is also important, chemical catalysis is reported to be cheaper than biological catalysis. But the modifications like using immobilized biocatalysts and the enhanced reuse can serve a competitive cost compared to the chemical catalysts [45].

Numerous approaches are still in progress for the optimization of the biodiesel production process adaptable to different feed types, compositions and downstream requirements. One of the novel processes mentioned in recent studies is the "Mcgyan Process" that consists of a continuous fixed bed reactor that can produce biodiesel using a metal oxide based catalyst. The process is an alternative method for continuous transesterification and can use a wide variety of feedstock, does not consume the catalyst, reduces the reaction time from hours to seconds, and does not use water or dangerous chemicals [16,46,47]. Treatment of the lipids extracted from *Dunaliella tertiolecta* and *Nannochloropsis oculata* with Mcgyan process to produce biodiesel reached up to 85% in the conversion of triacylglycerides and free fatty acids to alkyl esters [48].

Alternatively, by shifting the production route, microalgal oils can be used to produce "green diesel" by a process known as catalytic hydroprocessing. The basic difference of this with transesterification is the usage of hydrogen rather than the alcohol. Also the by-products like propane, water and carbon dioxide differ from glycerol of transesterification [8,16]. Green diesel has superiority over biodiesel with its lower oxygen constitution similar to the petroleum derived fuels with a near zero oxygen content. The primary goal of the processes is to produce a minimized oxygen containing green diesel with maximized final energy content [8,16]. Also because of the high paraffinic hydrocarbon content green diesel has higher cetane numbers and energy content on mass basis compared to biodiesel. Using the microalgal oils for green diesel will be advantageous because of the application potential in the conventional petroleum refinery systems with existing infrastructure and the by-products (H_2O and CO_2) can be recycled to be used in the microalgae cultivation again.

With the rapid progress in the area of genetic engineering the researchers can now present the genome sequences of microalgae for example, a lipid producing microalgae specie that has potential in biofuel production, i.e. *Nannochloropsis gaditana* genome is newly finalized and may become a model organism like *Chlamydomonas reinhardtii*, *Chlorella variabilis*, *Micromonas pusila*, *Ostreococcus tauri*, *Ostreococcus lucimarinus*, *Thalassiosira psudonana*, *Emiliana huxleyii*, *Fragilariopsis cylindrus*, *Aerococcus aophagefferens*, *Cyanidioschyzon merolae* and *Phaeodactylum tricornutum* with completed genomes [49,50]. By understanding these model microorganisms in molecular basis we can have the chance to improve productivities in future applications.

The ultimate idea was the excretion of the oils from the microalgae cells without the need of extraction step. This idea was supported with the studies focusing on *Escherichia coli* and *Saccharomyces cerevisiae* to excrete lipids through membranes with genetic engineering tools [51,52].

2.2. Biohydrogen

Hydrogen with its unique properties is accepted to be a renewable, sustainable and environmentally friendly energy carrier that serve as one of the most promising alternative solution to overcome the growing environmental concerns considering the future energy demands [53,54].

Today there are various conventional as well as novel strategies to produce hydrogen from fossil fuels (steam reforming, plasma reforming, thermal cracking, gasification), biomass (pyrolysis, microbial conversion, syngas conversion, supercritical conversion, gasification) and water (photolysis, thermolysis, electrolysis) incorporating chemical and biological processes [27,29,55]. Even if the hydrogen production is dominated by the chemical processes using fossil fuels, biological processes started to get more attention since the first studies in the 1920s [56–58].

The metabolic routes for biohydrogen production differ according to the microorganism [27,59]:

*Microalgae:
(a) Direct photolysis: $2H_2O \rightarrow light \rightarrow 2H_2+O_2$
(b) Indirect photolysis:
(1) $12H_2O+6CO_2 \rightarrow light \rightarrow C_6H_{12}O_6+6O_2$
(2) $C_6H_{12}O_6+12H_2O \rightarrow 12H_2+6CO_2$
*Photosynthetic bacteria:
(a) Photofermentation: $CH_3COOH+2H_2O \rightarrow light \rightarrow 4H_2+2CO_2$
(b) Water-gas shift: $CO+H_2O \rightarrow H_2+CO_2$
*Anaerobic bacteria: Dark fermentation:
$C_6H_{12}O_6+2H_2O \rightarrow 4H_2+2CO_2+2CH_3COOH$

Related with their photosynthetic productivity and light utilization efficiency, microalgae can perform special biochemical and photochemical reactions with minimum requirements that make hydrogen production possible under aerobic and anaerobic environments [60–62].

Microalga like Anabaena sp. [63,64], *Chlorella pyrenoidosa* [65] *Chlorella vulgaris* [66], *Platymonas subcordiformis* [66,67], *Spirulina platensis* [68] and especially *Chlamydomonas reinhardtii* [69,70] get attention in biohydrogen production.

Microalgae use two classes of oxygen sensitive metalloenzymes, nitrogenases and hydrogenases, that are closely related with the final biohydrogen generation process in photosynthesis [63,71].

There are several types of hydrogenases like the hup-encoded NiFe-uptake hydrogenases, hox-encoded NiFe-bidirectional hydrogenases, FeFe hydrogenases, NiFeSe-bidirectional hydrogenases

and Fe-only hydrogenases utilized by bacteria and microalgae. On the other hand nitrogenases of purple non-sulfur bacteria and heterocyst microalgae can be classified by their metal cofactor as molybdenum, iron and vanadium types [72,73].

Nitrogenase is the part of the biological nitrogen fixation and hydrogenase is capable to catalyze hydrogen consuming reactions. Biohydrogen production based on these enzymes differs in energy consumption. Hydrogenase mediated reactions are about three times more efficient than nitrogenase catalyzed reactions based on spent energy in the form of ATP. On the other hand nitrogenases are relatively less sensitive to oxygen compared to hydrogenases [74–76] Relatively or not, because both enzymes are sensitive to oxygen it is important to control culture conditions for optimal hydrogen production.

Microalgal biohydrogen production can take place during photosynthesis from water by the two photosystems, dark fermentation of the reduced carbon produced by photosynthesis and photofermentation by the enzymatic oxidation of intracellular reductants derived from fermentation [77–80].

Even if the studies on microalgal biohydrogen started in the 1930s [56,81,82], it was shown that the production can be achieved by the inactivation of photosynthetic water oxidizing activity, catalyzed by the reaction center of photosystem two (PSII) [83–85]; the productivities could not reach to meaningful levels until there was a novel protocol depending on sulfur deprivation [69]. This protocol depends on the succession of photosynthetic reactions in which oxygen production and carbon accumulation take place and anaerobic photoreactions through which the consumption of stored photosynthetic cellular metabolites for biohydrogen generation take place. Sulfur deprivation triggers the conversion of PSII centers to an intermediate in the PSII repair cycle, in other words from a QB-reducing to a QB-nonreducing form resulting in the decrease of photosynthetic oxygen evolution capacity [86–88].

Compared to the other methods like using inhibitors (DBMIB, DCMU, SAL, C1-CCP, etc.) or alternating light/dark cycle, the removal of sulfate from the growth medium achieves a reversible inactivation with sustainable and increased hydrogen production [83–85].

Most of the recent studies focused on the two stage sulfur deprivation protocol; however as summarized by some of the example studies from batch to continuous processes with various procedures the use of different microalgae is a good sign of the progress towards the future (Table 4). But again some microalgae like *Platymonas subcordiformis*, *Platymonas helgolandica* or *Chlamydomonas moewusii* are not sensitive to sulfur deprivation as well as *Chlamydomonas reinhardtii* so utilization of inhibitors and nitrogen starvation enhance the hydrogen production [89,90].

Also the outdoor studies point out another bottleneck in the productions. Even if the applications in lab scale are giving promising results outdoor cultures still cannot reach the comparable levels. Microalgae cells have problems to tolerate the light inhibition and sulfur deprivation when exposed to direct sunlight outdoors. They need to be acclimated to light before the hydrogen production phase [91].

Besides the studies on the enhancement of the biohydrogen production focusing on culture techniques like utilization of immobilized microalgae or stressed cultivations with regard to light and medium components, the progress in genetic engineering to improve production levels focuses on engineering truncated antennas of the species for better sunlight utilization outdoors without inhibition or engineering oxygen tolerant species that can produce hydrogen in aerobic conditions which is still under investigation [73,92].

2.3. Bioethanol

Bioethanol may be considered as an alternative clean burning fuel, because of its environmentally friendly combustion products with low greenhouse gas effect relative to fossil fuels. Today commercial bioethanol production is mainly from agricultural stocks and raw materials like corn, rice, wheat, cassava, sugar cane, sugar beet and sweet sorghum, through biochemical or thermochemical processes [16,17,93]. Even though the route of production depends on the raw material it can be summarized as the breaking of the biomaterial to simple sugars that further fermented using alcohol producing microorganisms. The produced ethanol later separated and concentrated through downstream processes (Fig. 4).

Facing the conflict of global energy and food demand, there have been increasing interest and worldwide studies in producing bioethanol from alternative sources, like microalgae rather than feedstock, that has minor impact in the daily life [16,17,93].

Bioethanol from microalgae can be produced through the fermentation of the microalgal biomass or directly through cellular reactions [94,95].

When considering ethanol production via fermentation the key is the accumulation of starch, which is a potential substrate that can reach half of the microalga's cellular dry biomass. Microalgal starch can be extracted by mechanical or enzymatic means, and further separated by downstream processes incorporating water or an organic solvent. This starch will be hydrolyzed to glucose to be used in ethanol fermentation [96,97]. Production mainly differs from other crop raw materials in the first steps of the process, because microalgae will need special concentration, harvesting and mechanical disruption systems related with their cellular physiology and culturing [98].

Microalgae can also excrete ethanol directly through the cell walls by means of intracellular processes under dark because illumination prevents the formation of ethanol, except for minor amounts. This process, which is reported in some microalgae like *Chlorococcum littorale* [99], *Chlamydomonas reinhardtii* [83,84], *Chlamydomonas moewusii* [100], *Chlorella vulgaris* [101], *Oscillatoria limnetica* [102], *Oscillatoria limosa* [99], *Gleocapsa alpicola* [103], *Spirulina platensis* [68], *Cyanoteche* sp. [99], covers the anaerobic metabolism in algae where the assimilation and formation of hydrogen, carbon dioxide, formate, acetate, ethanol, lactate, glycerol, and butannediol take place [17,83]. Degradation of intracellular starch, which is the main endogenous carbon source stored during aerobic phototrophic metabolism, to pyruvate is accomplished by the Embden–Meyerhof–Parnas and pentose phosphate pathways using pyruvate decarboxylase and alcohol dehydrogenase enzymes [99,102]. As stated by Gfeller and Gibbs [83], the lack of ethanol production by the microalgae cells during illumination is related with the absence of available reduced pyridine nucleotides, with the assumption of active acetaldehyde and alcohol dehydrogenases. Two possible conversion routes for acetyl-CoA to acetate and ethanol can be followed. In the former route, half of the acetyl-CoA is converted to acetate by a deacylase while the rest is reduced to ethanol with acetaldehyde as intermediate. In the latter route, acetyl-CoA is reduced to acetaldehyde that undergoes a dismutation sequence until the formation of acetate and ethanol [83,84].

Enhancing direct microalgal bioethanol production by genetic manipulations is also under consideration. Genes from the ethanol producing bacterium *Zymomonas mobilis* was transfered into microalgae *Synechococcus* sp. aiming to give the ability to utilize the fixed carbon for direct ethanol excretion to the culture volume through the cell walls [93,104].

The novelty of the process lies in the separation of produced bioethanol from the culture medium. This process eliminates the harvesting step that decreases the energy costs and water usage associated with the separation processes required for algae harvesting and fuel extraction [94,105].

Besides bioethanol being the main product, the remaining microalgal biomass may be used to produce biomethane again

Table 4
Microalgal hydrogen production.

Microalgae	Cultivation	Process	PBR type	Culture medium	Hydrogen production procedure	Time	H_2 production	Refs.
Anabaena variabilis (CCAP 1403/4B)	Continuous–photoautotrophic	Lab	Stirred (300 mL)	Nitrogen free Allen-Arnon	• Nitrogen free Allen-Arnon medium • Under vacuum for 15 min • Increased light intensity for 5 h at the 6th day of cultivation	35 days	12–14 mL g_{DW}^{-1} h^{-1}	[164]
Anabaena variabilis (ATCC 29413)	Continuous–photoautotrophic	Lab	Vial (14 mL)	Allen-Arnon (containing Na_2MoO_4 or Na_3VO_4 or neither)	Under argon flushing	80–100 h	Up to 5 nmol h^{-1} μg $Chla^{-1}$ at pH levels of 7–9 with Na_3VO_4 added cultures	[165]
Anabaena sp. PCC 7120 (3 hydrogenase mutants from PCC 7120)	Batch–photoautotrophic	Lab	Sealed polystyrene cuvettes (1 cm light path, 4.7 mL capacity)	BG 11	• Nitrogen free BG 11 medium • Under anaerobic conditions • Various light intensities	32–40 h	Up to 0.8–1 mmol per cuvette (4.7 mL)	[63]
Anabaena sp. PCC 7120 and its mutant AMC 414	Continuous–photoautotrophic	Outdoor	Tubular-coiled (4.35 L)	BG 11	• Nitrogen free BG 11 medium • Under argon atmosphere	7 days	14.9 mL $h^{-1}L_{PBR}^{-1}$ (373 mL total) with the mutant	[166]
Anabaena variabilis (ATCC 29413)	Batch–photoautotrophic	Lab	Panel (500 mL capacity)	BG 11	• Nitrogen free BG 11 medium • Under anaerobic conditions • Increased light intensity	50 h	Up to 40–50 mL	[167]
Chlamydomonas reinhardtii (CC124)	Batch–photomixotrophic, Synchronous and unsynchronous growth	Lab	Stirred glass bottles(1.2 L)	TAP	TAP-S medium (transferred by centrifugation)	140 h	• 102 mL (with 4 h synchronized cultures) • 86 mL (with unsynchronized cultures)	[168]
Chlamydomonas reinhardtii (Dang 137C mt+)	Batch–photomixotrophic	Lab	Stirred glass bottles (500 mL)	TAP	• TAP-S medium transfer by centrifugation • TAP-S medium inoculation by TAP culture (10% inoculation)		175 mL L^{-1} (with centrifuged cultures, under 20–40 μE $m^{-2}s^{-1}$ average light intensity)	[87]
Chlamydomonas reinhardtii (CC124)	Continuous–photomixotrophic	Lab	Stirred glass bottles (1050 mL)	TAP-S (90 μmol sulfate added)	• TAP-S medium, two stage chemostat with aerobic stage and anaerobic stage	4000 h	Up to 0.58 mL $h^{-1}L_{PBR}^{-1}$	[169]
Chlamydomonas reinhardtii (Dang 137C mt+ and a nonmotile mutant CC 1036 pf18 mt+)	Batch–photomixotrophic-immobilized	Lab	Panel (160 mL)	TAP (0.46 mM sulfate replete)	• TAP-S medium • TAP medium with limiting sulfate (10–20 mM) • Anaerobic conditions	23 days	45 mL day^{-1} (380 mL total)	[170]
Chlamydomonas reinhardtii (Dang 137C mt+)	Batch-photoautotrophic, mixotrophic and heterotrophic	Lab	Flat glass bottles PBR (1.5 L volume)	HS (CO_2 bubbling); TAP with or without CO_2 bubbling	• Sulfur free medium • Anaerobic	60–80 h	• 1.1 ± 0.4 mmol L^{-1} (~2.8 mL $(hL)^{-1}$) in photoautotrophic cultures • 4.5 ± 1.6 mmol L^{-1} (~4.0 mL $(hL)^{-1}$) in photoheterotrophic cultures • 0.9 ± 0.8 mmol L^{-1} (~6.9 mL $(hL)^{-1}$)in photomixotrophic cultures	[171]
Chlamydomonas reinhardtii (Dang 137C mt+)	Batch–photoautotrophic	Lab	Flat glass bottles PBR (1.5 L)	High salt	• HS sulfur free medium • Argon purging during the first 24 h of deprivation	100 h	• 71 ± 3 mL L^{-1} (175 μE $m^{-2}s^{-1}$ light intensity, pH 7.7) • 52 ± 2 mL L^{-1} (420 μE $m^{-2}s^{-1}$ light intensity, pH 7.4)	[172]
Chlamydomonas reinhardtii (CC124)	Semi continuous–photomixotrophic	Lab	Stirred tank (2.5 L)	TAP	• TAP-S medium • Various dilutions • Anaerobic conditions	127 days	1108 mL	[173]
Chlamydomonas reinhardtii (CC124)	Batch–photomixotrophic-immobilized	Lab	Vials (75 mL)	TAP	• TA-S-P (no sulfate, no phosphate) medium	160–180 h	0.30 ± 0.02 mol m^{-2} (12.5 μmol mg^{-1} $_{Chl}$ h^{-1})	[174]
	Batch–photomixotrophic	Lab	Glass bottles (325 mL)	TAP	• Alginate entrapped	192 h	• 3.95 μmol × 10^6 $cells^{-1}$ h^{-1} with TAP-S	[90]

Chlamydomonas reinhardtii (CC124)					harvested cells flushed with or without argon • Anaerobic conditions • TAP-S or TAP-N • DCMU mixed ethanol addition • Dark anaerobic fermentation		• 3.4 μmol × 10^6 cells^{-1} h^{-1} with TAP-N	
Chlamydomonas reinhardtii (CC124)	Batch–photomixotrophic	Lab	Stirred tank (1, 2.5,5 L)	TAP-S	• TAP-S medium transfer by centrifugation • Various mixing time • Various light energy • Scale-up	192 h	Max with 1.22 kJ s^{-1} m^{-3} light energy and 2.5 min mixing time: • 1 L PBR: 1.53 ± 0.05 mL L^{-1} h^{-1} • 2.5 L PBR: 1.32 ± 0.05 mL L^{-1} h^{-1} • 5 L PBR: 1.02 ± 0.05 mL L^{-1} h^{-1}	[202]
Chlamydomonas reinhardtii (CC124)	Batch–photomixotrophic	Lab	Tubular (110 L)	TAP-S	• TAP-S medium transfer by centrifugation • Modified with silica nanoparticle to enhance scattering	48 h	3121.5 ± 178.9 mL (0.6 mL L^{-1} h^{-1})	[203]
Chlorella pyrenoidosa C-101	Batch–photoautotrophic	Lab	Bubble column (650 mL)	Modified Bristol	Nitrogen flushing under dark for "> 20" h	60–65 h	6.9 × 10^{-2} m^3 kg^{-1} cell	[65]
Chlorella vulgaris MSU 01	Batch–photomixotrophic	Lab	Stirred (500 mL)	Modified BG11 and MJ	• Anaerobic conditions • Corn stalk as carbon source in the medium	6–7 days	26 mL	[175]
Platymonas subcordiformis	Batch–photoautotrophic	Lab	Serum bottle (295 mL)	Sea water medium	• Dark incubation • Anaerobic nitrogen atmosphere • Addition of specific effectors (DCMU, DCCD, DBMIB and CCCP) • Continuous illumination afterwards	8 h	0.339 mL h^{-1} L^{-1} (1.44 mL); based on 1 × 10^6 cells mL^{-1} at 15 μM CCCP added culture after 8 h of illumination	[66]
Platymonas subcordiformis	Batch–photoautotrophic	Lab	Serum bottle (295 mL)	Sea water medium	• Sulfur deprive medium • Incubation under dark • Anaerobic conditions • Continuous illumination afterwards	50 h	11,720 nL h^{-1} (with seawater-S medium)	[67]
Platymonas subcordiformis	Batch–photoautotrophic	Lab	Torus (1.5 L)	Defined mineral	• Dark incubation • Anaerobic nitrogen atmosphere • 15 μM CCCP addition • Continuous illumination afterwards	4–6 days	7.20 mL h^{-1} (236.6 ± 7.0 mL)	[176]
Platymonas helgolandica var. *tsingtaoensis*	Batch–photoautotrophic	Lab	Jars (130 mL)	F2	• Anaerobic • Illuminated -S medium • Dark CCCP/DCMU or both added medium	25 h	• 0.002 mmol L^{-1} with–S medium • 0.160 mmol L^{-1} with CCCP medium • 0.014 mmol L^{-1} with DCMU medium • 0.290 mmol L^{-1} with CCCP +DCMU medium	[89]
Spirulina platensis (NIES-46)	Batch–photoautotrophic	Lab	Erlenmeyer flasks (60 mL)	SOT	• Nitrogen free medium • Under dark	20 h	2 μmol mg$_{DW}$$^{-1}$	[68]

Table 4 (*continued*)

Microalgae	Cultivation	Process	PBR type	Culture medium	Hydrogen production procedure	Time	H_2 production	Refs.
					• Anaerobic conditions			
Synechocystis sp. PCC 6803 and its mutant NDH-1 complex deficient M55	Batch–photoautotrophic–encapsulated	Lab	Vials (2 mL)	BG 11	• Nitrogen atmosphere • Under cycled light and dark exposure	5 days	0.005–0.045 mM	[177]
Tetraspora sp. CU2551	Batch–photomixotrophic	Lab	Vials	TAP (with, or without, 0.5 mM β-mercaptoethanol for 24 h)	• Sulfur and nitrogen deprived medium • Argon flushed anaerobic atmosphere	> 24 h	17.3–61.7 μmol $mg_{chl\ a}^{-1}$ h^{-1}	[178]

through anaerobic digestion for electricity generation. The carbon dioxide and discharged water formed as by-products of the fermentation can be recycled for the microalgae cultivation to fulfill the biorefinery concept [94,105].

2.4. Biomethane

Today, agricultural plants or by-products are conventional sources for renewable biogas production, which is mainly a mixture of methane (55–75%) and carbon dioxide (25–45%), by anaerobic digestion. Biomethane from the biogas can be used as a fuel for transportation and electricity generation or for heating purposes especially in the rural areas [16,106,107].

Microalgae with their dominancy over crop plants by means of productivity per hectare triggered the attention for the production of biogas. The solar energy, converted and stored by the microalgae cells through photosynthesis, can be transformed into energy through the anaerobic digestion to produce methane [106,108].

Microalgal biomass, having high cellular lipid, starch and protein, low cellulose and on the other hand the absence of lignin, gives a reliable and effective anaerobic digestion. These specifications make microalgae a good alternative for effective biomethane production compared to the other crop plants [16,107,108].

Microalgal biomass can be processed anaerobically step by step with specialized groups of bacteria [109]:

- Hydrolyzation of biopolymers to monosaccharides by hydrolytic bacteria.
- Fermentation of the monosaccharides to carboxylic acids and alcohols by fermentative bacteria.
- Conversion of the acids and alcohols to acetate, hydrogen and carbon dioxide by acetogenic bacteria.
- Conversion of the end products of acetogenic reactions to methane and carbon dioxide by methanogenic bacteria.

Microalgal biomass remaining after the anaerobic digestion can be further processed to make fertilizers that can add an extra value to the overall process. Also the carbon dioxide portion of the produced biogas can be recycled again to cultivate the needed microalgal biomass for processing [107,110–112].

A predicted model of microalgae biomass for digestion to produce electrical and thermal energy discussed the economic profitability related with technical aspects of the production system. The results showed that the microalgae productivity, harvesting, concentration and usage of high rate anaerobic digesters are the key factors for the economic energy production [113].

A life cycle perspective study using a stochastic model indicated that, when compared with the conventional crops like switch grass, canola and corn, the environmental impact of microalgal biomass production is higher with respect to energy demand, greenhouse gas emissions and water use regardless of cultivation location. Only in overall land use and eutrophication potential microalgae present favorable results [114]. This may seem to be a disadvantage to produce biomethane from microalgal biomass but the studies also supported that the integrated processes using proper photobioreactors, combining microalgae cultivation and waste water treatment systems for biomethane production will help to reduce the environmental impacts resulting in a competitive and effective production [114,16,115].

2.5. Integrated processes

Relations between parameters and the target products (biomass, lipid, hydrogen, ethanol) are important in the selection of the microalgae for efficient biofuel production. As can be followed from the tables summarizing the microalgal biofuel productions,

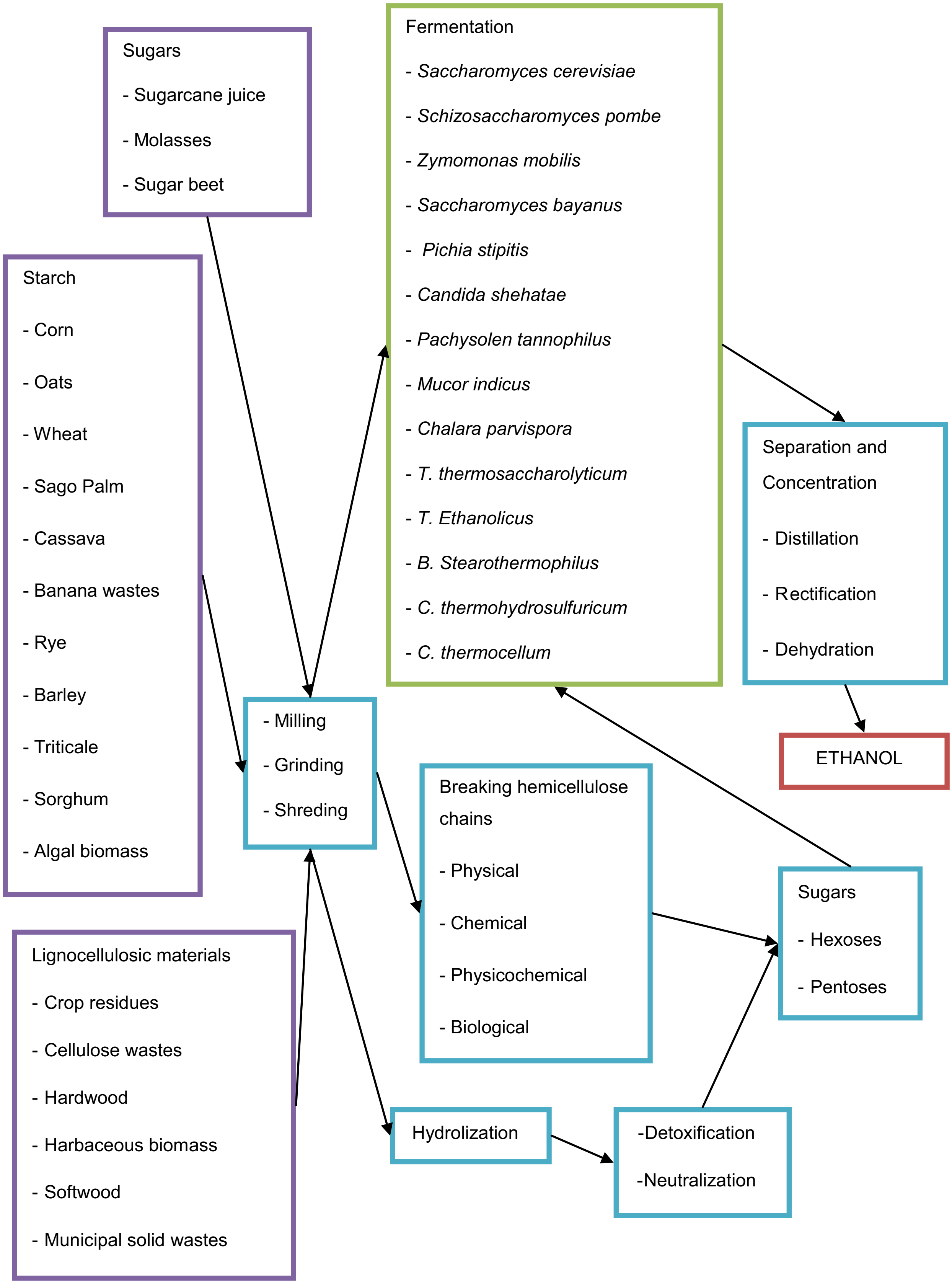


Fig. 4. Ethanol production from biomass production.
Source: Adapted from Refs. [93,98,201].

studies focus on the separation of production processes into two main steps: growth mediated and product mediated. When considering direct biofuel production the main approach is product mediated for higher lipid, hydrogen or ethanol production. When considering biofuel production integrated with other microorganisms (Table 5) the main approach is growth mediated for higher

Table 5
Integrated biofuel processes working with microalgae.

Microalgae	System	Product	Ref.
Chlamydomonas reinhardtii (UTEX 90)	Enzymatic treatment of algal biomass and conversion to ethanol by *Saccharomyces cerevisiae*	Ethanol	[179]
Chlamydomonas reinhardtii (UTEX 90)	Hydrothermal acid treatment of algal biomass and conversion fermentation by *Saccharomyces cerevisiae*	Ethanol	[180]
Chlorella vulgaris (IAM C-534)	Enzymatic treatment of algal biomass and conversion fermentation by *Saccharomyces cerevisiae*	Ethanol	[181]
Synechococcus leopoliensis (IAMM 6)	Fermentation of acid treated saccharified algal biomass by yeast Saccharomyces sake (IFO 2347)	Ethanol	[97]
Chlamydomonas reinhardtii, *Chlorella vulgaris* (macroalgae *Undaria pinnatifida*)	Enzymatic treatment of pre-acid hydrolyzed algal biomass fermentation by 4 different strains of *Escherichia coli*	Ethanol	[182]
Schizocytrium sp.	Fermentation of hydrothermal treated algal biomass by *E. coli* KO11	Ethanol	[183]
Microcystis aeruginosa and *Anabaena variabilis*	Hydrolysis and fermentation of Super Critical fluid pretreated algal biomass by *Saccharomyces cerevisiae*	Ethanol	[204]
Scenedesmus obliquus	Fermentation of algal biomass hydrolysate by *Kluyveromyces marxianus* IGC 2671, *Saccharomyces carlsbergensis* ATCC6269 and *Saccharomyces bayanus*	Ethanol	[205]
Anabaena sp. PCC7120	Fermentation of hydrogen produced residual algal biomass by *Enterobacter aerogenes*	Hydrogen	[184]
Arthrospira (*Spirulina*) *platensis*, *Nannochloropsis* sp. *and Dunaliella tertilecta*	Anaerobic fermentation of thermal pretreated algal biomass by immobilized *Clostridium acetobutylicum*	Hydrogen, acetone, ethanol, butanol	[206]
Chlorella vulgaris ESP6	Dark fermentation of acid or alkaline/enzyme pretreated algal biomass hydrolysate by *Clostridium butyricum* CGS5	Hydrogen	[207]
Chlorella sp.	Simultaneous hydrolysis and fermentation of algal biomass by sewage sludge consortia	Hydrogen	[208]
Thalassiosira weissflogii	Dark fermentation of algal biomass with the thermophilic bacterium *Thermotoga neapolitana*	Hydrogen	[209]
Chlamydomonas reinhardtii (IAM C-238), *Chlorella pyrenoidosa* (IAM C-212) and *Dunaliella tertiolecta* (ATCC 30909)	Fermentation of algal biomass by *Lactobacillus amylovorous* (ATCC 33620) and *Rhodobacter sphaeroides* RV	Hydrogen	[185]
Chlamydomonas reinhardtii (IAM C-238) and *Dunaliella tertiolecta* (ATCC 30909)	Fermentation of algal biomass by *Lactobacillus amylovorous* and *Rhodobium marinum* (A-501) mixed culture	Hydrogen	[186]
Chlamydomonas (MGA 161)	Fermentation of algal biomass by *Rhodovulum sulfidophilum* (W-1S)	Hydrogen	[85]
Chlamydomonas reinhardtii (C238)	Mixed cultivation of *Chlamydomonas reinhardtii* (C238) with *Rhodospirillum rubrum* (NCIB 8255)	Hydrogen	[187]
Scnedesmus sp.	Fermentation of algal biomass by anaerobic digested sludge	Hydrogen	[188]
Arthrospira maxima	Anaerobic digestion of algal biomass by sewage sludge consortia	Methane	[210]
Phaeodactylum tricornutum	Anaerobic digestion of algal biomass by potato factory origin anaerobic granular sludge	Methane	[211]
Scenedesmus sp.	Anaerobic digestion of high pressure thermal pretreated raw and residue (from lipid extraction) algal biomass and by anaerobic digestor sludge	Methane	[212]
Scenedesmus sp.	Anaerobic digestion of ultrasound and thermal pretreated raw algal biomass by sugar factory origin anaerobic granular sludge	Methane	[213]
Scenedesmus sp.	Anaerobic digestion of mild thermal pretreated raw algal biomass by sugar factory origin anaerobic granular sludge	Methane	[214]
Spirulina platensis, *Anabaena variabilis* and *Chlorella* sp.	Processing of algal biomass by an integrated system of methanogenic culture and *Rhodobacter capsulatus*	Methane and hydrogen	[189]
Spirulina platensis (UTEX 1926), *Rhodotorula glutinis* (2.541)	Mixed cultivation of microalgae and yeast using waste water	Lipid (biodiesel)	[190]
Spirulina platensis	Using excess CO_2 from the ethanol fermentation by *Saccharomyces cerevisiae*	Lipid (biodiesel)	[119]
Botryococcus braunii (two strains, BB763 and BB764), *Chlorella vulgaris*, *Chlorella pyrenoidosa*	Biodiesel production catalyzed by immobilized, *Penicillium expansum* lipase and *Candida antarctica* lipase B (Novozym 435)	Lipid (biodiesel)	[191]
Chlorella vulgaris (211/11B)	Anaerobic digestion of algal biomass by sewage sludge origin methanogenic culture	Methane	[112]
Chlorella sp. and *Scenedesmus*	Anaerobic digestion of algal biomass by sewage sludge culture	Methane	[106]
Chlorella sp.	Anaerobic digestion of algal biomass by sludge culture	Methane	[192]
Arthrospira platensis, *Chlamydomonas reinhardtii*, *Chlorella kessleri*, *Dunaliella salina*, *Euglena gracilis* and *Scenedesmus obliquus*	Anaerobic digestion of algal biomass by sewage sludge culture	Methane	[107]
Spirulina maxima	Anaerobic digestion of algal biomass by sewage sludge culture	Methane	[193]
Chlorella sp. and *Scenedesmus*	Anaerobic codigestion of algal biomass with waste paper by methanogenic culture	Methane	[194]
Tetraselmis	Anaerobic digestion of algal biomass by methanogenic culture	Methane	[195]
Chlorococcum sp.	Anaerobic treatment of distillery waste with algal biomass by acidogenic/methanogenic culture	Methane	[196]
Phaeodactylum tricornutum (CCAP1055/1)	Anaerobic digestion of algal biomass by granular seed sludge from digester treating potato processing waste water dominated by *Methanosaeta* sp. and *Methanosarcina* sp.	Methane	[197]

biomass production. The idea of integration is to unite each system in a way that both processes will complete each other in order to have a higher productivity and lower cost using all the sources including waste and excess streams.

A good example is the usage of bioethanol from renewable sources rather than using the petroleum based methanol for a greener approach and to complete the real biofuel idea. Compared to ethanolysis, toxicity, the risk of vapor explosion due to methanol's low boiling point, lower oxidative stability, higher iodine value, and higher exhaust emissions are other motivations behind ethanol usage. Fatty acid ethyl esters have similar properties to methyl esters and the ethanolysis reaction can also work with various feedstocks utilizing catalytic and non-catalytic reactions like conventional methanolysis [116,89,117]. The cost will be the main limit but biorefinery concept can overcome the constraints.

Other than the direct bioethanol usage for biodiesel production, the integration of large scale microalgal cultivation with ethanol biorefineries to use the excess CO_2 to grow microalgae, which can then be used as feedstock for biofuel production, is also noteworthy. An economic evaluation study for Iowa, USA, pointed that selecting a good candidate strain like *Chlorella vulgaris* can be feasible and profitable when integrated with bioethanol refineries [118]. A similar approach for the utilization of excess CO_2 from ethanol fermentation was investigated to be feasible for the production of *Spirulina platensis* for lipid production [119] and keeping in mind that the residual algal biomass can be recycled again to the fermentation process, this integration will be promising. Also the integration of the microalgae production systems with sugar mill facilities will help to share the excess CO_2, water, molasses and energy from the boilers that will be advantageous for the process feasibility [120].

Considering the environmental impact co-culturing microalgae with methane oxidizing bacterial communities utilizes the excess CO_2 allowing a methane oxidation with minimum emission. On the other hand the produced oxygen through photosynthesis lowers the need of external O_2 to maintain methane oxidation, by 55%. This approach will have potential for large scale anaerobic waste water plants which will reduce two important greenhouse gases CH_4 and CO_2 in a single process [121].

Valorization of the excess glycerol from the biodiesel and CO_2 for glycerol carbonate production is a good alternative for the economic benefit of biodiesel production [122].

An economic study indicates that the microalgae residues from biodiesel production that will be used in biomethane production for electricity generation will make the production of biodiesel from algae more competitive by reducing the overall production costs especially energy needs for downstream processes [123].

Using microalgae for waste water bioremediation targeting biofuel production has potential for daily life application [124]. A conceptual design of Green Wisdom Inc., USA, using microalgae for integrated bioremediation and biofuel production showed the potential of microalgae for rural communities' economic acceleration and sustainability. A successful integration can decrease biofuel costs and valuable microalgal by-products may help the production costs to be competitive. On the other hand using CO_2 and waste water the microalgae can improve the environmental load with regard to pollution and achieve more sustainable living [125].

2.6. Light to fuel

Starting with strain isolation, microalgae proceed through different process steps until the final product is obtained (Fig. 5). Even if these steps are well adapted in commercial scale processes based on biomass production for some species [2,3,5,17], microalgae production still needs development for the key processes such as pretreatment, production modes, photobioreactor design, downstream techniques and energy demand to be more effective, sustainable, productive and environmentally friendly [4,18,19,74,114,115].

Amongst the immense species of microalgae there is an opportunity for finding a candidate having a potential to be used for biofuel. Also contrary with the diversity, few strains of focus is another factor that increases the possibility. Realization of the biofuel production depends on the specie which should be selected according to the target product. Focusing on the target which is microalgal biofuel production, the process from light to fuel can be divided into two levels: laboratory and industrial.

Laboratory is the main unit where necessary investigations from culture conditions to design can be done with multiple experiments that simulate the effects of different parameters like light, mixing, temperature and aeration on the production to get realistic potential of the microalgae with regard to biofuel production. Targeting the industrial scale all the data starting with the test tubes should be transferred step by step to flasks and later to photobioreactors where online control of the process can be handled and possible modifications can be done to increase the production. Being of diverse types, photobioreactor is a tool for scale-up that should be investigated in detail considering all the pros and cons [2,126–129]. Photobioreactors will help to mimic interactive scenarios faced with the larger systems in a controlled environment ensuring an objective comparison among all types. On the other hand in the downstream processes another key feature of the microalgal fuel process can be investigated in laboratory scale to evaluate their application, efficiency and harmony with the whole process. Again various applications and methods for separation, concentration, extraction and purification can be compared to have a higher productivity in industrial process [4,18,21,72]. After the detailed investigation considering all the steps from the test tube to the photobioreactor stage with the entire downstream process one can have a clear idea about the potential of the microalgae for biofuel production.

The bridge from laboratory to industry is the scale-up that plays a vital role in commercialization. There are different strategies for the scale-up depending on various factors. The idea is to determine a constant value for a parameter of a unit volume that is assumed to represent the whole system which is in other words independent from the scale. These factors include operational parameters like mixing time, gas transfer rates, dissolved gas concentration, mixer blade tip velocity, linear velocity, light intensity per illuminated area, light intensity per volume, Reynolds number, and Power number and dimensional parameters like illumination area culture volume ratio, height to diameter ratio, and blade diameter to tank diameter ratio [2,3,72,73,76,91,129]. Especially in the case of photobioreactors to develop a successful scale-up strategy it is necessary to consider both operational and dimensional parameters. Again a strong background investigation on laboratory basis will warrant a successful scale-up.

After the development of the scale-up strategy the laboratory knowledge is ready to be transferred to large scale cultivations. Because large scale productions have extra complexity for management related with the increased dimensions of the systems they may need different methods of approach to solve the problems compared to small laboratory productions. Entire process from photobioreactors to downstream systems must be scaled up maintaining the harmony for high productivities.

Other than the physical part of the system design increase in scale will also necessitate the economical investigation of the process [45,113]. Even if the economy seemed to be shaded in laboratory scales it is the actual motivation of the whole process that targets a valuable product to have an economic benefit. A successfully scaled-up process should also be feasible and

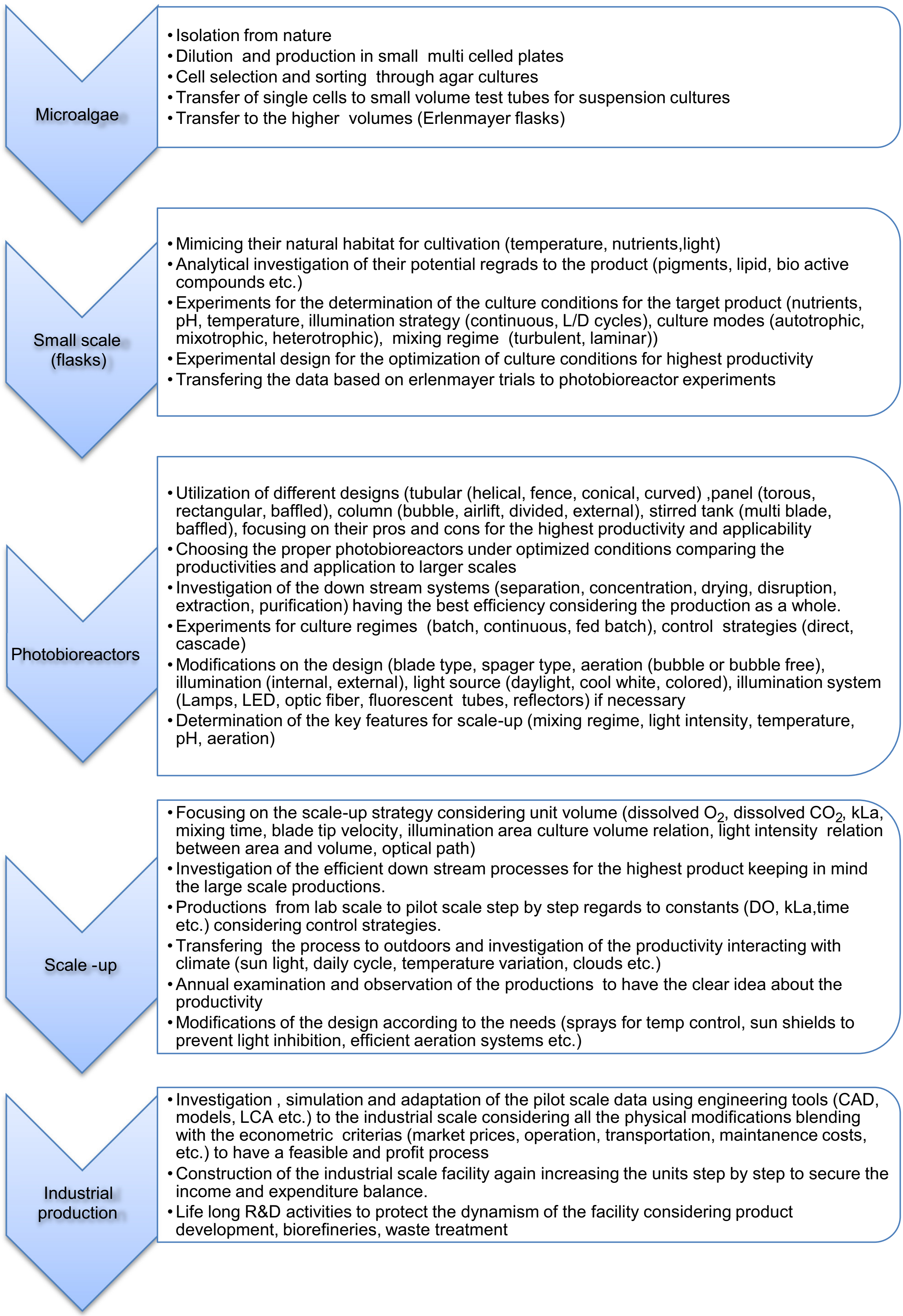


Fig. 5. From light to bioproduct considering the basic process steps.

profitable as well as have high productivity through a well designed system based on the knowledge from laboratory to the outdoors.

2.7. Future prospects

Focusing on the light to fuel concept the main concerns about the future are based on the bottlenecks of microalgae productions which can be surpassed with the advantages that are pointed out by various researchers [7,9,114,126–134]:

- Requirement of large areas for outdoor scales with considerable amount of water. This problem can be solved by the cultivation on lands like desert, arid and semi-arid lands using saline, brackish water or coastal seawater.
- Limited species of interest. Because they are research friendly with the possibility of laboratory scale production there is a chance for rapid investigation of new species. Using photobioreactors culture conditions can be controlled for the needs of the studied microalga in order to understand its specifications and potential. This way especially the limited outdoor productions based on environment and contamination tolerant species can be spread to other species.
- Limited outdoor applications in large scales with regard to systems and dependency on weather. The background knowledge about their biomass targeted large scale open pond cultivations and development of diversified new designs of photo-bioreactors considering CO_2 feed, illumination and mixing efficiencies and preventing evaporation losses will accelerate the applications. Also because the microalgae can be transferred using conventional pipelines, mass productions can be done in areas with proper climate and transferred to other places for further applications, which may lower the production costs especially in the case of biofuels.
- Nutrient requirement like phosphorus that is becoming scarce. Especially the utilization of waste waters and by-products from other conventional processes like flue gases, organic carbon containing wastes may help the production to be feasible and sustainable.
- Need progress in downstream processes to lower the production costs. Working on the new separation and extraction systems with higher efficiencies gives promising results for future applications.
- Unclear cost per volume compared to fossil fuels with regard to a market price. Considering their faster growth compared to terrestrial plants, their higher biomass productivity on areal basis, and a better adaptation to elevated CO_2 and nutrient levels, microalgae has a high potential to be produced in vast amounts with appropriate selection of the species and production systems. Modification of their biochemical composition by simple changes in their cultivation conditions (nutrients, light intensity, temperature, mixing etc.) for higher productivities of the targeted products is another advantage. On the other hand their ability to synthesize and accumulate various high value products (e.g. biopolymers, proteins, polysaccharides, pigments) will help to reduce the production costs and support the feasibility. Keeping in mind their useful adaptation with the biorefineries, high potential for carbon credits and efficient blending with liquid fuels, operational costs besides the production costs can be decreased to competitive prices. The progresses in material science and technology will also help producers utilize the proper equipment with lower cost.
- Low production levels with regard to biofuels to compete with the fossil fuels can be solved with the developed production and downstream systems to some point. Also the progress in genetic engineering to understand the cellular production mechanisms and the modifications in molecular basis is promising for future scenarios. On the other hand utilization of photobioreactors increases the productivities of various species by controlling the culture conditions and eliminating contaminations.

However the key is to get realistic projections without exaggerating the potential. Because of this, microalgae based biofuels should be objectively handled to have a clear vision of the future. Main challenge is the realization of the microalgal biofuel usage considering present and future targets (Table 6).

Today several turnkey applications are presenting the dynamic progress of the microalgal energy. Algenol Company aimed to eliminate the separation and extraction costs and had promising results from patented process for ethanol secreting microalgae using outdoor photobioreactors without harvesting and killing the microalgae just someway milking it. Also Origin Oil another company has patented a novel single step extraction process in which the biomass, water and oil is separated continuously without chemicals. The company is now transferring the knowhow for large scale productions. Keeping in mind the integrated applications NASA with its OMEGA project has planned to produce microalgae offshore in large sea farms utilizing elastic bag type photobioreactors. This way the land costs will be limited, the microalgae cultured with waste water and flue gases will help to prevent pollution and using osmosis technology cleaned water will be separated from microalgae that will help for the feasible production of biofuels, fertilizers and food. Joule Energy genetically engineered a microalga like microorganism to secrete lipid hydrocarbons in a single step process eliminating all downstream processes and producing ready-for-energy biofuel.

Microalgal biofuels may not become a direct competitor to fossil fuels in near future but can be an alternative at some areas. According to EIA liquid fuels will continue to dominate the world marketed energy use. In future scenarios when considering oil prices the change will mainly affect the consumption of liquid fuels which are the key features in transportation [1]. This can be a good opportunity for microalgal biofuels where they can find a lane to grow. Microalgae based biodiesel and bioethanol have the potential to be used directly or as a blend for internal combustion engines, while biohydrogen can be used in green diesel production or in the fuel celled vehicles, which will orient the microalgal biofuels to our daily life. On the other hand their potential to be used as a support with the conventional fuels is an important route for the economic benefit. Also to have feasible investment and production costs the contribution for improved biofuel production by specialized strains with higher yields, which are applicable for outdoor cultivations and integrated processes with other microorganisms, serves a realistic strategy for sustainable production. To reach the goals for the future, economy of today should be considered.

3. Economy

The main concern of the economy, which is also its driving force, can be summarized as the balance of the massive contestation between the earth's limited resources and the humans' endless appetite for progress sometimes far more than the needs. From the bioeconomy point of view with regard to microalgal fuels, this can be depicted as the interrelation between the cost of the production and the revenue from sale keeping in mind the competitiveness with other fossil fuels.

To produce all microalgae based fuels one should first produce the microalgae. The road to feasibility passes from the well-established and analyzed production costs related with

Table 6
The constraints, future targets and comments-opinions for microalgal biofuel realization.

Focus	Today	Target	Comments/opinions
Microalgae	Few strains in focus, variable productivities, more interest from other sectors like pharmaceutical, aquaculture etc., rather than energy	Selection of new strains, and routes for maximized productivities with regard to biofuel	Genetically modified strains can have a chance for higher productions but the key is to control their possible effects on nature and determination of their applications with legislations. New species will also be discovered and used in researches for progress. Also rather than the monoculture processes, mixed or co-cultures of microalgae with other microorganisms will increase the chance of application
Photobioreactors	Limited large scale productions, high investment costs depending on the technical and constructional needs	Improved designs considering outdoor productions interactive with environment, lower production cost	Outdoor open ponds will be used in the future but with the improving materials and construction techniques, they can be transformed to closed systems with feasible modifications for increased productivity with diverse species. On the other hand two basic designs tubular and panel will also find commercial application as a support unit that may be used for more sophisticated by products to decrease the production cost of the biofuels produced by pond systems
Microalgal biofuels	Limited usage but increasing interest with the progress in fuel cell technology, engines, hydrogen storage, microalgae oil/ethanol production and extraction, several production steps	Higher productivities with single step processes, leading to daily life usage in areas like transportation	Considering its potential, biodiesel will be the dominator over the other microalgal biofuels. But keeping in mind the bioethanol and biomethane production through integrated processes they can be a support for the economic feasibility of the biodiesel process by using its residues and recycle streams. Biohydrogen can be classified separately with its potential to be used in fuel cells
Biorefineries	Beginning of the spread usage with biorefinery concept	Stronger biorefinery attitude with the corporation of residential and industrial facilities supported with production of biomass and biofuels as by-products	Like petroleum refineries gaining all the possible products will be an advantage for microalgae that has diverse by-products in addition to biofuel important for different sectors. The key is the integration with other conventional bioprocesses like fermentation, digestion and waste treatment
Economy	Variable feasibility depending on the production process and technology	Feasible production considering improved downstream processes, new photobioreactor designs and carbon credits	Other than the economic profit from the biofuel, producing by-products through biorefinery concept will help to decrease the operational costs. Possible integration with other commercial processes excess emissions, energy and waste can be used in the productions serving as an economic advantage. Also transporting microalgae through conventional pipelines can be an advantage for the production in suitable climates and refinement of the raw biomass in specified facilities elsewhere will decrease the cost of downstream processes. This way the producers can send their product to centralize refinement facilities to eliminate extra infrastructure similar to the refineries in the petroleum industry. Microalgae based biofuels will also act as a backup to control the prices of especially petroleum when the reserves start to deplete resulting in big price sway with regard to elevated oil concession and drilling costs
Energy	Limited share in renewables and limited daily life usage	Higher share, widespread usage	Energy from coal, hydro and nuclear will continue to dominate the massive demand. But depending on the petroleum prices microalgal biofuels can increase their share especially in the area of transportation. Biodiesel and bioethanol will get the main attention as blends with petroleum whereas biohydrogen with fuel celled cars

technology, which also serve a fertile ground for future research and development for new industries. Based on the main cost components that add up to the total cost, a process should consider the cost of land (yard improvement, roads etc.), production systems (buildings, service facilities, storage silos, open pond or photobioreactor, inoculation, nutrient delivery, control, sterilization, cooling, heating, pumping, aeration and mixing units), harvesting systems (centrifuges, filters, settling tanks, conveyors, dryers etc.), downstream systems (homogenization, disruption, extraction and purification units) and operational expenses (management, maintenance, labor, electricity purchase, insurance, employee training, transportation, consumables and taxes) [215–222]. Any progress to decrease the cost in these components, where research and development expenses are also important, is a challenge and motivation for researchers and entrepreneurs.

The key is to have an objective estimation of the unit cost of production in order to foresee the market value. The economic background of production mainly built on the conventional markets of health food, feed and valuable chemicals especially related to cosmeceuticals or pharmaceuticals. The cost of production with regard to these markets is in the range of 1–7$ kg^{-1} of raw algal biomass [5,128,223,224] depending on the production process. But these values may rise up to 1000$ kg^{-1} (2) especially in the case of utilizing sophisticated systems, including photobioreactors and downstream equipment, for example in the productions related with pharmaceutical industry. However one should also consider that the revenue of such a high cost will also pay high. For example some biochemicals have high price like "astaxanthin" from *Haematococcus* has a price up to 10.000$ kg^{-1} or "β-carotene" from *Dunaliella* has a price up to 3.000$ kg^{-1} depending on the purity and quality in the world market [225].

Similar to the conventional markets the main point in the cost of fuel production as mentioned, is also related with the process. For microalgae the challenge is to compete both with fuel crops and fossil fuels with regard to the unit cost.

Considering the case of biodiesel or green diesel, according to the average prices for April 2013 [226] algal oil which can be used should not exceed the prices of its competitors: vegetable oils (palm, soybean, rapeseed, sunflower), with an average density of 0.91 kgL^{-1} [227], around 0.7–1.3$ L^{-1}, petroleum (West Texas, Dubai, Brent) around 0.59–0.64$ L^{-1} and petroleum diesel around 0.76–0.85$ L^{-1}. Blending these selling prices with the cost of microalgae biomass production, Chisti (5,128) foresaw a cost of 0.34$ kg^{-1} as a limit for feasible production yielding a cost of 2.80 $ L^{-1} for algal oil. This assumption depending on the 55% oil content per biomass weight is still realistic for today to compete petroleum diesel because of the relative productivity of the species. Other than the importance of the selected species the average cost of microalgal oil under compatible conditions (with an oil content of about 30% of biomass, free CO_2, low cost of nutrients and 1.14 L kg^{-1} oil extraction capability) is estimated as 2.95$ L^{-1} in open ponds while it needs to be enhanced to 3.8$ L^{-1} in the photobioreactors [228] to reach the feasible limits.

On the other hand similar approaches for market feasibility can be accepted for ethanol which has a price of 0.67$ L^{-1} or for hydrogen production based on water electrolysis that has an average value depending on the electricity price, 3–11.8$ kg^{-1} also [226,228].

On the economic aspect, the utilization of open ponds or photobioreactors and the downstream steps should be well evaluated. A case study with tubular type photobioreactor to produce high value intracellular oils from marine microalga *Phaeodactylum tricornutum* showed that the key points for a feasible production are based on the production and recovery of the biomass, extraction of oils from the wet biomass and purification of the crude extract. The average cost of producing biomass was 32.16$ kg^{-1}, the crude esterified oil was 396.52$ kg^{-1} while this cost stepped up to 4602$ kg^{-1} after purification. This huge difference marked an overall cost profile of 40% that resulted from biomass production and 60% from recovery [215]. To overcome these high costs different scenarios of biofuel production with regard to integrated processes aiming wastewater treatment and biofuels production showed that the best cost will be around 302 $ $barrel^{-1}$ which is still very high compared to petroleum [216]. Another cost pricing study estimates a value microalgal oil (1 barrel = 42 US gal) to be 356$ $barrel^{-1}$ for open pond and 760 $ $barrel^{-1}$ for photobioreactor productions. On upgrading to green diesel via hydrotreatment these costs will elevate to 413$ $barrel^{-1}$ and 863$ $barrel^{-1}$ [217] which are still far beyond even the average selling prices of petroleum diesel which are about 122–135 $ $barrel^{-1}$.

Despite all these values microalgal biofuels can still be the vital solution for the replacement or support to fossil fuels. The companies are continuously investing millions of dollars for research and realization. After delivering 80.000 L of algal diesel to US navy in 2010, multi-million dollar company, Solazyme has another contract of about extra 550.000 L. Also they have announced in March 2013 the results of consumer survey as the feedback of a partnership with a leading fuel retailer, that 92% of the consumers are interested in using algae based biodiesel again in their vehicles instead of fossil fuels considering environmental issues. Also another company Sapphire has scaled up its process to 100 acres by 2013 targeting a capacity of 1 million barrels of crude algal oil per year in a full 300 acres facility. On the other hand Synthetic Genomics has a deal with Exxon for about $600 million for the realization of motor fuels from algae back in 2009. This immense amount was planned as the key for development. According to Bloomberg Business in 2013, Exxon is still interested in the business even if the venture was prolonged to 25 years rather than their first prediction of 10 years, till commercialization.

These update news are prosperous for the future of microalgae based fuels even if there is still some time to feasibility. Nevertheless knowing inevitable reality of the vanishing fossil fuel reserves in the next century and the conflict of fuel crops competing with food crops, microalgae that have the ability to utilize the colossal energy of the sun will have a clear future.

4. Ethical issues

Similar to all other fuels from raw to product the ethical issues are also important for the microalgal biofuels. The need for a better world for humanity with regard to environment and economy, without omitting fair and secure distribution of energy, is the motivation for the bioenergy and bioeconomy. The key to take a strong step to realization depends on the ethical values considering human rights, justice, solidarity, sustainability and stewardship [229–231]. To fulfill the ethical needs, microalgae based biofuels have some advantages over other conventional fuels. First of all they will not interfere with the food and water that are essential for humanity, with their ability to live in various environments. They are environmentally more sustainable with regard to their ability to utilize sun, even flue gases and waste waters. This will also help to reduce the net greenhouse gas emissions to limit global warming besides water treatment. They can be adapted and integrated with conventional fuel refineries, infra-structure and trade principles in order to have a smooth transform to a real bioeconomy. Last but not the least, with their various by-products and production systems, microalgae can act as leverage for labor, inter-generational equity and resilience in the society [229–233].

On the other hand again similar to the other fuels the main concern is the controlled progress in order to minimize the environmental impacts especially with regard to production land. Consumption of space may cause problems with the local habitat. This risk will be higher if alien species or genetically modified species are utilized. Also a vicious cycle will be formed if the petroleum based fertilizers and gases will be utilized without optimization causing a continuation in petroleum dependency. Another risk of using fertilizers is the runoff of the culture process streams resulting in uncontrolled eutrophication in water systems. These points should be well focused for prevention because they will contradict with ethical issues that will damage the reliability of the microalgal fuels. On the global basis the maturation of the industry should be well coordinated and controlled not to face any chaos. Also to prevent monopolization research and development should be public focused and respect human rights fulfilling the concept of fair trade [229–231].

We have to think for today and tomorrow considering the next generations, and the shared welfare and equity will be related with the diversification of the energy sources and reducing the dependency on fossil fuels in which microalgae will be a promising candidate.

5. Conclusions

Combining the background knowledge of their biology and culture production, microalgae diversity producing various valuable bioproducts is an advantage. Also the commercial interest with the rising awareness for environmental and energy issues are strong catalysers for the progress.

Today we come to a point where nothing seems to be solved in a quick and easy way. Environmental problems versus energy struggle force us to choose a pathway for sustainable future. One way is to continue on the vast consumption of natural resources thereby increasing the problems. Another way is to change our old habits and focus on the sustainable and environmentally friendly lifestyle starting with the consumption of energy.

Biofuels, started with the first generation continuing with the second and the third and leading today to the fourth generation focusing on the modified organisms with low carbon impact, will continue to evolve with the changes of the world needs and priorities.

Based on the economic consideration, microalgae production systems and facilities are required to be well planned for a feasible production strategy. Even though it seems to face cost crisis, marketing and commercialization hardships for today's world, the relief in the ethical issues related to microalgae biofuel production starts a new era for clean, sustainable and environmental friendly production for future.

Even if microalgal biofuels seem to be a rookie compared to the fossil fuels, their baby steps may have a chance to get faster only if we can analyze their potential realistically.

In the attempt to have a better world, microalgae contrary to their name may have a macroimpact on progress.

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